The molecular mechanisms of reaction wood induction

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Chapter 4
The Molecular Mechanisms of Reaction Wood Induction

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4.1 Introduction

Reaction wood (RW) develops in stems and branches in response to the perception of endogeneous and environmental stimuli caused by a change in the natural position. As a result the stem or branch bends back towards its original position. It is generally stated that in most cases, in gymnosperms RW is formed on the lower side of branches and bent stems and is called compression wood (CW). In angiosperms it is formed on the upper side of branches and bent stems and is called tension wood (TW). Wood formed on the other side of branches and bent stems is called opposite wood (OW).

It was commonly believed until the end of the 1980s that RW was induced by the stress state of the new wood layer at cambium vicinity. The bottom of a branch should be in compression thus promoting CW in gymnosperms, the top being in tension should promote TW in angiosperms. For experimentation the challenge was to submit the external layer of a living stem to high tensile or compressive axial stress without any other signal such as gravity or light, and without too big a

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physiological stress due to the mechanical loading. Fournier et al. (1994) showed that the cross section grows while it is loaded. For each material point, the superposition of stress and strain begins from the time the material is differentiated. That comes from the obvious assumption that "a tissue cannot be loaded before it exists". The main consequence is that the new wood layer does not contribute at all to support the load of the existing trunk or branch and the resulting support stress should be zero at the stem periphery. A change of paradigm therefore had to be made: it is not the stress that induces RW formation but the RW formation that produces different stress levels (in tension or compression) in the new wood layer.

Very often, successive growth events are used by trees "to solve" some mechanical problem, in addition to building of the prescribed structure, in order to restore the posture of an inclined tree (Thibaut et al. 2001; Moulia et al. 2006), to search for the light, to change the tree architecture after death of a major axis, and so on. RW is a solution for a drastic and sudden change in the existing wooden structure of the tree. It is commonly used by all trees, particularly in the juvenile stage. RW is created very locally in answer to a global mechanical problem for the tree by creating a step change in the pre-stressing state of the new layer. According to modelling simulations, the curving efficiency of asymmetrical stressing of the axis using RW is nearly five times higher than the best solution using normal wood asymmetry alone (Almeras and Fournier 2009).

Solving the mechanical problems of a tree through growth is possible because of the flexibility in growth of the meristematic tissues in the length or ramification (primary growth) and the diameter (secondary growth) of each axis (trunks and branches). This structure needs to be mechanically sound and able to respond to most hazards faced by the tree. In the tree, each elementary growth event has to be precisely managed: action or dormancy, rapid or slow cell division, cell differentiation and cell wall formation. And each of these events has mechanical consequences. In addition, the new growth events that involve primary and/or secondary growth seem to be coordinated at the whole tree level. RW originates from cambial activity (secondary meristem), which adjusts the number of cell division to modify the axis diameter and in particular its second moment of area, as well as the proportion of fibres and the cell wall thickness to modify the density and the mechanical properties of the new layer. Through cambial activity the microfibril angle (MFA) in the S2 layer of the cell wall is also adjusted to modify the axis flexibility both by changing the modulus of elasticity for a given tissue density and the maximum allowed strain before damage (more flexibility appears to be an adaptation to wind); this may also modify the pre-stressing state of the new wooden layer. Last but not least, cambial activity adjusts the chemistry of cell wall components to modify the pre-stressing state of the new wooden layer; this may be done in conjunction with the adjustment of MFA (these changes are discussed further in Chaps. 2, 3, 5 and 6).

Hence, the following questions are raised concerning the induction of RW through modulation of cambial activity. What are the different external or internal signals related to secondary growth in order to solve different mechanical requirements? Where are the perception sites for the new mechanical requirements for the

tree? Could a signal get to particular cells in the cambium in order to manage new growth? If such a signal exists from the perceptive cells to the cambium, what about the conversion of the perception into messages transmitted to the secondary meristem? What is the process of "regulation"? How are these messages transcribed in the making of RW? Also most of the questions raised for RW formation could also be addressed to the regulation of primary growth since both primary and secondary growth must be coordinated at the whole tree level.

In this chapter, after reviewing different kinds of signals (gravity, light, mechanical strain) that can induce a mechanical reaction causing RW formation, we will focus on the molecular mechanisms that might be involved in the perception and response to gravity and other mechanical stimuli. Since it is quite clear that the signal perception gives way to synthesis of proteins guiding the production or translocation of various plant hormones, we will review their implication in the gravitropic or phototropic mechanical response inducing the making of RW. We will also discuss the insights provided by global approaches such as transcriptomic, proteomic and metabolomics, made possible by the sequencing and annotation of the genome of trees such as poplar and eucalyptus. In particular, these global approaches gave new information on genes involved in RW formation.

4.2 Perception and Signal Transduction

4.2.1 Physical Parameters Inducing RW

To maintain a branching architecture that is optimal for growth and reproduction, plant stems continuously control their posture to counterbalance environmental physical parameters such as gravity, wind and light, that shift their orientation from the vertical (Moulia et al. 2006). In trees, this postural control has been mainly studied in response to gravity (Du and Yamamoto 2007). In the primary growing zone of stems, reorientation of woody plant organs involves local differential elongation growth between opposite sides of the stem. In stem parts undergoing radial growth, sectors of RW are produced that can be associated with eccentric cambial growth. In angiosperm woody species, TW is often characterized by fewer vessels and the formation of fibres with smaller diameter containing a gelatinous layer inside the S2 layer of secondary cell walls where cellulose microfibrils are aligned into a vertical orientation (Fig. 4.1, Mellerowicz et al. 2008; Chap. 3) with a lower lignin content (Pilate et al. 2004). In conifers, CW is characterized by tracheids with a thicker secondary cell wall than in normal wood, with higher lignin content, intracellular spaces at cell corners, and a realignment of cellulose microfibrils in the S2 layer orientation with respect to the axis of the stem (Timell 1986). These differences in secondary cell wall biochemical composition and architecture of RW generate internal growth stresses in the stem (Chap. 5), and because of its

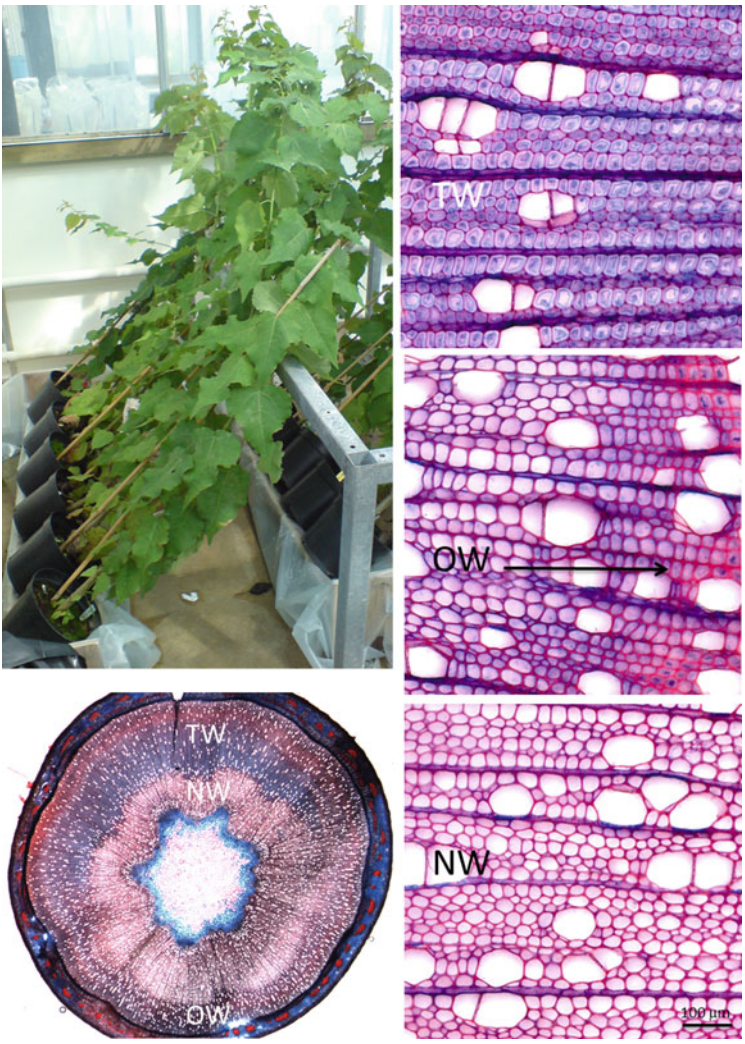

Fig. 4.1 Induction of TW by tilting of hybrid aspen trees (*Populus tremula* × *tremuloides*). After 17 days of tilting, a band of TW (TW) is seen on the upper side of the tilted stems. The mature normal wood (NW) produced before tilting, and the opposite wood (OW) produced at the lower side of the stems are shown at higher magnification on the right side of the figure. The sections were stained with safranin and alcian blue. Picture courtesy of Ewa Mellerowicz

unilateral formation in the stem, it induces a directional movement, bending the stem towards a favourable position.

As discussed recently by Felten and Sundberg (2013), many experiments were performed where branches or stems are tilted, bent into complicated shapes, grown on c1inostats or centrifuges, to identify if a single stimulus is responsible for the induction of RW. For loop experiments on shoots or branches, the localization of

RW suggested that its induction depends rather more on positional sensing (sensing of the local angle of the growing organ relative to the gravitational field) than sensing of mechanical stresses such as tensile or compressive stresses (Spurr and Hyvärinen 1954).

However, RW formation is not only induced by gravitational stimulus. TW has been reported to form in the vertical stems of rapidly growing poplar (*Populus*) trees (Telewski 2006). RW was also observed in branches and stems contributing to crowns reshaping after loss of apical dominance (Wilson and Archer 1977) and is part of the mechanism allowing up-righting of apricot tree stems in response to increased shoot and fruit load (Almeras et al. 2004). Plant exposure to wind spray or to repeated stem bending to mimic the wind triggers (1) transitory inclination of the stem, but with a duration of stem inclination much shorter than the presentation time necessary to observe the induction of RW (Jourez and Avella-Shaw 2003) and (2) mechanical signals such as stresses and strains. Exposition of poplar stems to repeated transitory bendings produced a flexure wood with anatomical similarities to TW (Pruyn 1997; Pruyn et al. 2000). In *Abies fraseri*, the morphology and function of wood developed after daily flexures (<20 s) were more closely related to CW than normal wood (Telewski 1989). CW was also detected in wind-treated *Pinus* (Berthier and Stokes 2005). One of the best examples demonstrating that RW is not exclusively induced in response to positional sensing came from analysis of the kinematics of stem straightening (for review, see Moulia et al. 2006; Moulia and Fournier 2009). The characterization of the spatio-temporal curvature field during stem straightening allowed the recognition of a biphasic pattern: an initial phase of spatially homogeneous up-curving due to gravitropic response and a second phase of stem decurving that propagates basipetally to finally reach a steady state where the curvature is concentrated to the base of the growth zone. In some plants, this decurving phase occurred before the shoot apex had overshot the vertical, demonstrating that this phenomenon was not due to the perception of the inclination angle, and was called autotropism. This autotropic decurving has been observed in many plants (Moulia et al. 2006). Studying this process in poplars (*Populus nigra* × *Populus deltoides*), Coutand et al. (2007) observed that no part of the trunk overshot the vertical during stem straightening. Indeed, during the initial phase of up-righting, arcs of RW were detected homogeneously all along the upper side of the trunk, whereas a second sector of RW was produced on the initially lower side in the most distal part of the trunk, contributing to this autotropic decurving. Recently, Bastien et al. (2013) studied the gravitropism kinematics of different organs from 11 angiosperms by time lapse photography, including both primary elongation zones and zones of secondary growth in which active bending is achieved through the production of TW. The biphasic pattern of tropic reactions described above was found to be generic whatever the type of the organ, and it was shown to lead to a steady state shape in which the apical part is straightened whereas the curvature is more concentrated at the base of the stem. However, inter- and intra-specific variability occurred in the steady states and in the transients: whereas some plant organs reached a steady state without overshooting the vertical, others exhibited oscillations around the vertical axis. Bastien et al. (2013)

also demonstrated that the minimal dynamic model cannot involve only gravisensing but the simultaneous sensing of the local curvature, referred by the authors as proprioception. When the organ is tilted and straight, gravisensitive control is dominant and the organ bends up actively. However as curvature increases, the inclination angle decreases and proprioceptive control takes over and autotropic counter-bending is produced starting in the apical parts of the organs, allowing it to straighten and align with the gravity vector. These data also suggest that the different shapes observed along the straightening response reflected a different ratio between graviceptive and proprioceptive sensitivities.

Another physical parameter that can influence reorientation of the stem by RW formation is light interception. Because of their co-occurrence under natural conditions gravi- and phototropism are intrinsically linked (Correll and Kiss 2002). Remarkably, phototropism sensing converges to common molecular actors with gravitropism and notably auxin transport and perception (Hohm et al. 2013). Typically, stems and stem-like organs have positive phototropism and negative gravitropism. Additive or synergetic effects were reported (Kern and Sack 1999) making the identification of their relative contribution and their possible interactions problematic. To address this issue, gravity effects were experimentally reduced or abolished (e.g. microgravity, space flight) while applying directional light source to induce phototropism (Millar and Kiss 2013). To date, it remains technically difficult to alleviate or reduce gravity effects on trees. The few possibilities offered to researchers consist in the manipulation of gravity orientation by tilting potted trees in combination with directional light modifications. Matsuzaki et al. (2006) report phototropism in trees submitted to different gravitational stimulations as observed on mountain slopes. Basal parts of the trees did not show bending in response to tropisms, which was limited to upper parts of stems. The authors suggested that reorientation could be achieved by asymmetric radial growth due to the formation of RW as is the case for gravitropism and further proposed trees inclination on slopes depends on phototropism. The same team later proved that the mechanism involved in phototropism required differential xylem production (Matsuzaki et al. 2007). In a recent study, Collet et al. (2011) studied long-term (4 years) phototropic response of *Fagus sylvatica* and *Acer pseudoplatanus* after canopy opening in natural conditions. Plants reacted by righting themselves towards the light source and this involved reorientation of the lignified parts of the stems through asymmetrical formation of RW. Herrera et al. (2010) noticed changes in the orientation of apical part of pine seedlings but not in the basal parts even after 22 days of light and gravi-stimulation. Although limited to primary growth, this work provided molecular data on the interaction of these two tropisms, scarce for tree models. Interestingly, photo- and gravitropic responses of potato plants were different depending on the time of the day suggesting they were also regulated by an endogenous circadian clock to some extent (Vinterhalter et al. 2012). Such complexity, far from being completely understood in herbaceous plants, still needs to be established in trees where secondary growth in reaction to phototropism and gravitropism is still a matter of exploration.

All these data converge on the induction of RW during plant postural control. Clearly the triggering of RW formation during tropic reaction not only is related to the sensing of the inclination of the stem versus gravity but also involves curvature sensing. TW has been shown to systematically be formed on the lower side of the branch when autotropism dominates gravitropism, allowing for curvature compensation (Coutand et al. 2007). Similar shifts in the location of RW along the tropic motion have also been described for CW in conifers (Sierra de Grado et al. 2008). But, how these diverse physical parameters (gravity, local curvature, light) are perceived by plant cells in order to induce RW remains unclear. Are all these physical parameters perceived by a common sensing mechanism or is there any crosstalk at a later stage during the signaling pathway?

4.2.2 *Molecular Mechanisms Involved in the Perception of Mechanical Stimulation Leading to RW Formation*

In case of a gravitational stimulus, the resulting physical forces can deform or move objects of specific mass inside the cell. Two hypotheses are currently favoured: (1) the amyloplast-sedimentation in specialized cells named statocytes and the perception of the direction of this sedimentation and (2) the weight of the protoplasm itself triggering mechanical deformation of subcellular structures such as membrane, cytoskeleton elements and cell wall (Baluška and Volkmann 2011).

The role of starch-filled amyloplast sedimentation during graviperception is well documented in *Arabidopsis* (Morita 2010). In young shoots, statocytes are localized in the endodermis layer surrounding vascular tissues. These cells are highly vacuolated and equipped with prominent F-actin bundlets (Morita et al. 2002). The studies of different mutants affected either in starch formation (*pgm*) or in intracellular components such as the vacuolar membrane (VM) or actin microfilaments (AFs) that both modify cytoplasm viscosity and activity showed that amyloplast dynamics are important during shoot gravisensing (for review, Hashiguchi et al. 2013). Recently, by using a centrifuge microscope to analyse gravitropic mutants in *Arabidopsis*, Toyota et al. (2013) confirmed the importance of amyloplast movement perception during shoot gravisensing. In woody species, amyloplast localization in endoderm cells has been observed (Nakamura 2003) in the young shoots of Japanese flowering cherry tree (*Prunus spachiana*) and in young poplars (*Populus tremula* × *alba*) (Fig. 4.2a–d). However, a link between amyloplasts sedimentation and RW formation has not yet been demonstrated. Moreover, the endoderm is disrupted by secondary growth. In cross sections of older poplar stem, lugol-stained starch grains are observed in whole bark tissue as well as in the wood rays (Fig. 4.2e).

The cellular mechanism underlying curvature proprioception is unknown (Bastien et al. 2013). The sensing of cell deformation (strain sensing) or more precisely of the deformation of some cellular component is a good candidate

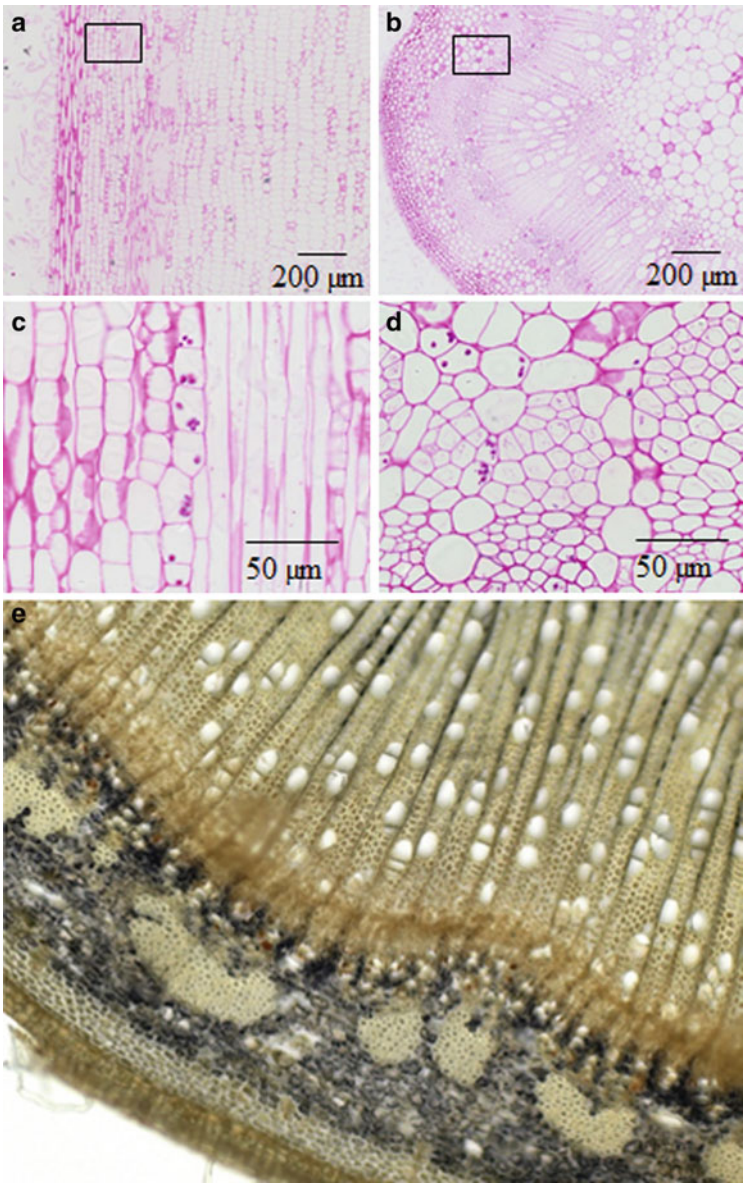

Fig. 4.2 Sections of poplar stems (*Populus tremula* × *alba*). Photographs (**a**–**d**) represent sections from the fourth bottom internode of a young plant with 20 developed internodes. Longitudinal sections (**a**, **c**) or transversal sections (**b**, **d**) were stained using Periodic acid/Schiff (PAS) reaction to detect starch and polysaccharides. Photographs (**c**) and (**d**) are, respectively, enlarged views of the photographs (**a**) and (**b**) (*black rectangle area*). Photographs courtesy of Wassim Azri. (**e**) This picture represents a cross section of a poplar stem, 40 cm under the apex. The tree height was 2.35 m and the diameter of the section is 5.3 mm. The freehand cut was stained by lugol. Photographs courtesy of Kevin Tocquard

(Wilson and Archer 1977; Bastien et al. 2013). Moreover the perception of the deformation of cellular components is suggested also through amyloplast sedimentation, in the gravitational pressure model (Baluška and Volkmann 2011), and through wind sensing (Moulia et al. 2011). A few molecular candidates have been identified as mechanoperceptors of this deformation. In trees, these are only putative. Results obtained from the herbaceous model plant *Arabidopsis thaliana* suggest molecular candidates at the CPMCW (cytoskeleton—plasma membrane—cell wall) continuum. They would be able to sense the mechanical signal from the plant cell wall and convert it into a molecular signal in the cell (Baluška et al. 2003; Telewski 2006).

The first molecular candidates are mechanosensitive (MS) ion channels. MS ion channels are membrane proteins that allow the transit of ions through cellular membranes. They open either directly by the force applied on the membrane or indirectly through links between the channels and/or both the cytoskeleton and cell wall components (Haswell et al. 2011). To date the involvement of MS ion channels in the perception of mechanical signals has yet to be clearly established. Nevertheless, several pieces of evidence show that ionic changes occurred after mechanical signals (Haley et al. 1995). In plants, MS ion channels (Cl^-, K^+ and Ca^{2+}) have been identified at plasma membranes using patch-clamp electrophysiology (Cosgrove and Hedrich 1991; Ding and Pickard 1993; Haswell et al. 2008). Currently three main groups of MS ion channels have been described in plants. First, the mechanosensitive channel of the small conductance (MscS) family from *Escherichia coli* which releases osmolytes from the cell (Booth and Blount 2012) has ten homologues (MSL1–10) in the *A. thaliana* genome (Pivetti et al. 2003). MscS homologues may release osmolytes in response to membrane tension and may be modulated by additional signals (Monshausen and Haswell 2013). The second Mid1-complementing activity (MCA) family is structurally unique to the plant kingdom. The MCA proteins are located in the plasma membrane and promote calcium influx upon mechanical stimulation (Nakagawa et al. 2007). Finally, the Piezo proteins are a class of MS cation channels which respond to mechanical stimuli (Coste et al. 2012; Kim et al. 2012). In *Arabidopsis*, there is a single Piezo protein (Coste et al. 2010; Kurusu et al. 2013; Monshausen and Haswell 2013) but it has yet to be characterized.

Other molecular candidates are receptor-like kinases proteins (RLKs) that are a family of proteins with an extracellular domain, a single transmembrane region and an intracellular cytoplasmic kinase (Shiu and Bleecker 2001). Among RLKs, wall-associated kinases (WAKs) are the most well-studied potential cell wall status receptors (He et al. 1999; Verica and He 2002). They have a particular interest because WAKs extracellular domains are able to bind to the cell wall (He et al. 1996). Notably, Wagner and Kohorn (2001) showed that *At*WAKs covalently bind the cell wall pectins, in a calcium-induced conformation (Decreux and Messiaen 2005). Moreover, a reduction of *WAK* expression inhibited cell elongation and altered morphology (Lally et al. 2001; Wagner and Kohorn 2001), indicating an activity in growth control. Therefore, WAKs are interesting candidates as sensors of cell wall integrity. Another candidate among RLKs for sensing

the cell wall integrity is the *Catharanthus roseus* RLK1-like subfamily (*Cr*RLK1L). The 17 members present an extracellular malectin-like domain (Lindner et al. 2012). Malectin proteins bind to glycoproteins in animal endoplasmic reticulum (Qin et al. 2012). The hypothesis that the malectin-like domain of CrRLK1L proteins binds cell wall polysaccharides or glycoproteins in plants has been proposed (Monshausen and Haswell 2013). In this subfamily THESEUS1 (THE1), a particular member with a plasma membrane location, could be a candidate for cell wall integrity sensing in *Arabidopsis*. THE1 was identified at a suppressor screen of cellulose-deficient mutant *cesA6* (Hématy et al. 2007). The *the1* mutant partially restored the hypocotyl elongation of the *cesA6*. However, mutations or over-expression of the *THE1* gene did not exhibit any effects in *Arabidopsis*. Consequently, THE1 was therefore proposed as a sensor of the cell wall status and modulator of cell elongation during perturbed cellulose synthesis.

Members of ArabinoGalactan proteins (AGPs) bind pectins and are also hypothesized to be cell wall integrity sensors. Indeed, AGPs are highly glycosylated proteins located in the cell wall (for more detail, see Chap. 3). Some AGPs bind cell wall pectin (Serpe and Nothnagel 1995) and could be attached to the plasma membrane via GPI anchors which would mediate intracellular signaling (Humphrey et al. 2007).

4.2.3 Mechanical Signal Transduction: Secondary Messengers

Regarding secondary messengers, few data are available in trees and almost lacking in the context of gravitropic stimulation and/or stem bending. However, herbaceous plant model data can be a starting point for future studies on tree models. According to Toyota and Gilroy (2013), calcium is an important and ubiquitous cell secondary messenger. Its specific role as a secondary effector in MS signaling has been extensively investigated in *Arabidopsis* and *Nicotiana* (Knight et al. 1991, 1992; Haley et al. 1995; Plieth and Trewavas 2002; Toyota et al. 2008). Gravity stimulation of *Arabidopsis* seedlings indicated a cytosolic Ca^{2+} concentration $[Ca^{2+}]_{cyt}$ increase with a first sharp increase followed by another less intense but longer signal (Plieth and Trewavas 2002). Wind also induced $[Ca^{2+}]_{cyt}$ increase in *Nicotiana* and *Arabidopsis* seedlings (Knight et al. 1992; Haley et al. 1995; Plieth and Trewavas 2002). Experiments conducted on trees support the involvement of Ca^{2+} in RW formation. By using a Ca^{2+} chelator (EGTA) or a calcium channel inhibitor ($LaCl_3$) which allowed modification of Ca^{2+} availability, CW formation was inhibited in *Taxodium distichum* gravistimulated stems (Du and Yamamoto 2003). The involvement of calcium in TW induction has also been suggested by indirect data obtained from several global approaches (see Sect. 4.4) notably through regulation of protein accumulation such as calreticulin, a Ca^{2+} storage

protein (Azri et al. 2009). The overall results suggested Ca^{2+} ion as a second messenger in the early stages of mechanical signal transduction.

Other secondary messengers have been identified as important after mechanical/gravitational stimulation. Azri et al. (2009) suggested the involvement of reactive oxygen species (ROS) in poplar TW formation induced through the accumulation of glutathione-dehydroascorbate reductase (GSH-DHAR), glutathione S-transferase (GST) and thioredoxin *h* (Thr *h*) proteins. Azri et al. (2013) further showed an induction of the *Thr h* gene in response to gravitropic stimulus. With an immuno-chemistry approach, they co-located Thr *h* proteins with amyloplasts in stem endoderm cells, thus providing a coherent framework for graviperception. More evidence from herbaceous models demonstrates that an interplay of ROS and Ca^{2+} could mediate mechanosensing: ROS can stimulate $[Ca^{2+}]_{cyt}$, and an increase of $[Ca^{2+}]_{cyt}$ can also lead to a ROS production via NADPH oxidase (Mori and Schroeder 2004).

Molecular candidates to convert the transient ionic Ca^{2+} signal to long-term biochemical signal are mainly calmodulins (CaM) and Ca^{2+}-dependent protein kinases (CDPK). Calmodulins are Ca^{2+}-dependent regulation proteins linking calcium to MS. CDPK are cytosolic proteins with a kinase domain, an autoinhibitory domain, and a calmodulin-like domain that bind calcium ions (Hrabak et al. 2003). In poplar, the expression of calmodulin encoding genes is up-regulated as soon as 10 min after a transient stem bending (Martin et al. 2009). Although very likely, a role for these proteins in the Ca signaling after mechanical stimulation still needs to be demonstrated.

Phosphatidylinositol signaling is another part of the gravitropism sensing network (Strohm et al. 2012). Membrane phospholipid Phosphatidylinositol 4,5-bisphosphate (PIP2) releases inositol 1,4,5 triphosphate (IP3 or InsP3) after phospholipase C (PLC) hydrolysis. InsP3 is accumulated during gravitropic response which later is repressed through PLC inhibition (Perera et al. 2001). InsP3, like Ca^{2+}, is a second messenger suggested as triggering intracellular calcium flow from vacuole (Allen et al. 1995).

Taken together, these results indicate that complex and interactive signaling pathways are involved after mechanical/gravitational stimulation. To date, no study has yet addressed these mechanisms in tree stems. Numerous questions remain to be resolved to understand the induction of RW. Why RW is produced in only one side of the stems, whereas the physical parameters triggering RW production are potentially perceived on both sides of the stem. Are the putative receptors uniformly localized in cells? Furthermore, if the endodermis cells are considered as sensitive cells, how is the inductive signal transmitted to cambium cells or developing xylem cells to modify the secondary wall composition and architecture? What cells act as gravisensor in older stems? How and where does crosstalk between gravisensing and proprioception occur?

4.3 Signal Transmission to Reaction Wood-Forming Cells

4.3.1 Hormone Action and Reaction Wood

The anatomical and biochemical features of RW have been the subject of extensive studies, which are described and reviewed in Chaps. 2 and 3. However the physiological and molecular signals inducing the formation of RW remain poorly described. Several plant hormones have been implicated in the regulation of cambial cell proliferation activity and in wood cell differentiation (for review, see Elo et al. 2009; Nieminen et al. 2012; Ursache et al. 2013). Until recently, their role in RW formation was studied through the application of exogenous compounds, the hormones themselves, their antagonists or transport/perception inhibitors. These experiments proved to be very informative as a first step towards deciphering whether hormones have the potential to induce RW formation. However, the possibility to genetically transform poplar and the use of high throughput molecular technics are, and will be continue to be, of great help in confirming and completing these observations.

4.3.1.1 Auxin

The application of auxin antagonists or transport inhibitors on both gymnosperm and angiosperm tree stems led to the assumption that auxin plays a role in RW formation. Increased amounts of auxin could induce CW formation in gymnosperms while a deficiency in auxin was required to form TW in angiosperm (for review, see Du and Yamamoto 2007; Felten and Sundberg 2013). However, such experiments do not prove the function of auxin under natural conditions because of the risk of uncontrolled modification of auxin homeostasis. To be validated, they need a better documentation of *in planta* auxin concentrations/amounts. Reports on the relationship between endogenous auxin levels and the formation of CW or TW are scarce and sometimes contradictory. For example, while Wilson et al. (1989) did not find a clear and conclusive correlation between the occurrence of CW and auxin concentrations, Funada et al. (1990) working on *Cryptomeria japonica* and Du et al. (2004) working on *Metasequoia glyptostroboides* found a higher amount of endogenous IAA (indole-3-acetic acid) in the cambial region producing CW. More recently, Hellgren et al. (2004) conducted a kinetic survey of endogenous IAA distribution across the cambial region of Aspen (*P. tremula*) and Scots pine (*Pinus sylvestris*) trees after bent-stem gravistimulation. These experiments showed that RW could be formed even when the pattern of IAA distribution in the cambial region is unmodified. The authors concluded that modulation of the auxin concentration gradient across the cambial zone might not be the signal that maintains the cells in an RW developmental state.

Data found in the literature are contradictory and were obtained on different species with different techniques and at different time points after stimulation. It is

therefore difficult to give a clear model of auxin role in RW formation. However, an involvement of auxin in the early steps of the induction process has not yet been ruled out. Actually, several reports showed that auxin signaling is responsive to gravistimulation. After 6 h of stem bending, the expression of two *AUX/IAA* genes in poplar (*P. tremula* × *tremuloides*) (Moyle et al. 2002) and one in *Eucalyptus* (Paux et al. 2005) was altered in TW compared to normal wood in unbent trees. In yellow poplar (*Liriodendron tulipifera*), a species that does not produce a typical G-layer, the expression of ARFs and AUX/IAA genes as well as other auxin-related genes is modified in TW compared to OW (Jin et al 2011). Similar results were also obtained in poplar (*P. tremula* × *tremuloides*) after 3 weeks of TW induction by leaning the stem (Andersson-Gunneråas et al. 2006).

High throughput approaches have already helped in improving our knowledge of auxin function in TW formation. Coupling these with functional genomic approaches could help to gain a better understanding of the role of auxin in TW formation.

4.3.1.2 Ethylene

The gaseous hormone ethylene has long been known to be produced in response to diverse stresses including mechanical solicitations (for review, see Braam 2005; Telewski 2006; Du and Yamamoto 2007) such as bending and tilting. These stresses usually stimulated wood production by increasing cambial activity and sometimes led to the production of RW. Like auxin, ethylene involvement in RW formation was first investigated by measuring ethylene or its precursor (ACC: 1-aminocyclopropane-1-carboxylic acid), and by using application experiments. For example, in the vascular cambium of *Pinus contorta* Dougl. ssp. *latifolia* branches, endogenous ACC was detected in association with CW differentiation, but not with OW (Savidge et al. 1983). Applications of ethrel, an ethylene releasing compound, stimulated wood production and were able to modify anatomical features of xylem in gymnosperm and angiosperm trees (Du and Yamamoto 2007). In angiosperm, although some of these features, like fewer and smaller vessels, can be reminiscent of TW characteristics (Little and Savidge 1987; Du and Yamamoto 2007; Love et al. 2009), it has to be noted that there is no report of G-layer induction by ethylene treatments.

Molecular approaches have helped to gain new insights about regulation of TW formation by ethylene. In bent poplars (*P. tremula* × *tremuloides*), a clear induction of *PttACO1* (ACC oxidase the last enzyme in the ethylene biosynthesis pathway) expression and relative activity were observed in the TW-forming tissues (Andersson-Gunneråas et al. 2003). Therefore, PttACO1 may represent a major control of ethylene asymmetric production during TW formation. In poplars leant for 3 weeks, the expression of genes related to ethylene signaling was also modified in TW compared to OW (Andersson-Gunneråas et al. 2006). In *L. tulipifera*, such modifications were seen as soon as 6 h after bending of the stem (Jin et al. 2011). Love et al. (2009) combined the use of ethylene-insensitive trees, ethylene-

overproducing trees, and the application of the ethylene-perception inhibitor MCP (1-methylcyclopropene) to explore ethylene physiological function in gravistimulated poplars. They showed that ethylene could be responsible for the stimulation of cambial cell divisions on the upper TW-forming side of leaning stems. In 2013, Vahala et al. identified 170 gene models encoding ERFs (ethylene response factors) in the *Populus trichocarpa* genome. Among these, 17 had a minimum of a twofold induction of expression in TW compared to normal wood. Over-expression of some of them in poplar resulted in anatomical or wall chemistry modifications that are reminiscent of TW features.

Ethylene and its signaling pathway seem therefore to control part of the molecular and physiological modifications underlying RW formation, especially the asymmetric increase in radial growth. However, it seems that the establishment of the full characteristics of RW involves ethylene in combination with yet unidentified other signaling factors (Love et al. 2009; Vahala et al. 2013; Felten and Sundberg 2013).

4.3.1.3 Gibberellins

Gibberellins (GAs) constitute another group of plant hormones known to promote secondary growth and xylem fibre length (Eriksson et al. 2000; Mauriat and Moritz 2009; Gou et al. 2011). Applications of exogenous GAs or GA inhibitors to tree stems can only provide indirect evidence for a role of GA in RW formation. In gymnosperms, the possibility of a role of GAs in CW formation has not yet been clearly demonstrated. However, experiments conducted on upright or tilted angiosperm trees helped to establish a correlation between GA and TW formation. For example, it has been shown that the application of GA to vertical stems of *Fraxinus mandshurica*, *Quercus mongolica*, *Kalopanax pictus* and *Populus sieboldii* induced the development of TW with typical G-fibres in the absence of gravistimulus (Funada et al. 2008). When tilted *Acacia mangium* seedlings were treated with GA their negative gravitropsim was stimulated. On the contrary, when they were treated with paclobutrazole or uniconazole-P, inhibitors of gibberellin biosynthesis, the gravistimulated upright movement of the acacia stems was inhibited and the formation of TW was suppressed (Nugroho et al. 2012, 2013).

No functional genomic experiment has yet proved that gibberellins could control RW formation. However, the use of a natural weeping mutant of *P. spachiana* brought some evidence for a role of GAs in TW formation. Exogenous application of GAs on branches of these Japanese cherry trees (*P. spachiana*) stimulated cambial growth and promoted TW formation on the upper side of branches resulting in an upright movement (Nakamura et al. 1994; Baba et al. 1995; Yoshida et al. 1999).

Together these results indicate that GAs seem to be involved in RW formation. However, more direct supporting evidence and a better understanding of the involved signaling factors is still needed to make a clearer conclusion.

As mentioned above, although sometimes quite indirect, an important amount of data indicates a role for auxin, ethylene and gibberellins in RW formation. This holds especially true for angiosperms since knowledge obtained on CW is less advanced. On the contrary, no such relation has been identified for cytokinins, abscissic acid or brassinosteroids. Most of the experiments designed to gain a better understanding of the role of plant hormones in TW formation focused on a single hormone, studied independently. It is, however, important to keep in mind that many hormones have been shown to interact with each other in a synergistic or inhibitory manner. For example, GA is known to stimulate polar auxin transport (Björklund et al. 2007) and IAA to promote ethylene biosynthesis (Abeles et al. 1992). Although the studies made so far have greatly improved our knowledge of RW biology, the use of high throughput molecular technics combined with functional genomics has started to, and should in the future, help to gain a deeper understanding of the processes underlying RW development. Moreover, hormones are currently mostly regarded as upstream primary responses to TW sensing (Felten and Sundberg 2013), but studies on hormone distribution and transport are still too scarce and contradictory to rule out the possibility of their involvement in the transmission of the signal from the perceptive to the RW-forming cells.

4.3.2 Other Candidates for Signal Transmission to Reaction Wood-Forming Cells

miRNAs are small non-coding RNA molecules (about 21 nucleotides) which cleave or degrade messenger RNA targets. In plants, they are involved in the regulation of a large number of physiological processes (Jones-Rhodes et al. 2006) through the targeting of cell metabolism, signal transduction and stress response mRNAs. Different authors (Griffiths-Jones et al. 2008; Lu et al. 2005, 2008; Zhang et al. 2010) have characterized mechanical stress-responsive miRNAs in *P. trichocarpa*, especially miRNA that were differentially regulated by bending. The predicted target genes encode transcription factors and proteins involved in various cellular processes. For example, the function of the target of miR1446 is a gibberellin response modulator-like protein and the target of miR160 is an auxin responsive factor. Although a direct link between miRNAs and RW has never been proven, the above-mentioned data indicate that these small molecules could be good candidates to explore the molecular network controlling RW formation. To do so, further genome-wide identification of miRNAs using a different experimental design (inclination) is needed, as well as functional characterization of the identified miRNA and corresponding targets.

Recently, several authors reported intercellular signaling by miRNAs and showed that some can move from one cell to another or over long distances (for review, see Marín-González and Suárez-López 2012). Since signaling from the cells that perceive the RW-generating stimuli to the RW-forming cells may require

long distance regulation of gene expression, it is tempting to consider miRNAs as good candidates for the signal transmission from perceptive cells to RW-forming cells.

4.4 Insights from Global Approaches

Despite the economic impact of RW occurrence in industrial process and its importance from a tree developmental point of view, the molecular mechanisms involved in the perception and response to the gravitational stimulus have not been extensively studied. Furthermore, very few studies have addressed this question by global approaches, which require the genome of the studied tree species to be sequenced and annotated.

Investigating the induction of RW is also a very complex question RW is formed very locally in answer to a global mechanical problem for the tree. In addition most of the regulations used in RW formation (division rate, cell elongation, cell wall thickening, MFA setting) are also used for normal wood formation. Experimental setups have to take into account this point to specifically address the question of RW induction. Most of the studies on genes or proteins acting as regulators of RW making were done on inclining experiments. By inclining the whole tree system by an angle of around 30° and letting it grow afterwards (see Chap. 5) a pure, long-lasting RW formation is induced at the base of the stem (see Fig. 4.1). At inclination angles of this magnitude there is a strong perception of disequilibrium, secondary growth processes are very active and no new primary axillary growth is observed. This is in contrast to very inclined (nearly horizontal) trees, which use growth through axillary buds (i.e. primary growth) to create new vertical axes.

4.4.1 Transcriptome Analysis

Transcriptomics of RW is still in its infancy. Quite limited reports are available although studies have been conducted for about two decades. Regarding the vast majority of transcriptomics works on normal wood, the reader should refer to the most recent review of Zhong and Ye (2013). Early work addressed gene expression during RW formation through target genes approaches, leading progressively to recent transcriptome-wide overviews. Tools for deciphering gene involvement in the control of RW formation are becoming increasingly available and although most recent gene expression measurement tools, such as RNAseq, are still under-used in this research field, hopefully this will change in the near future.

Most studies of angiosperm RW formation using transcriptomics have been conducted with poplar species and less frequently with *Eucalyptus grandis*, *Eucalyptus globulus* and *Eucalyptus nitens*. Other angiosperms species have been rarely studied with the exceptions of *A. thaliana* and the tulip tree (*Liriodendron* sp.). In

gymnosperms, most studies have been conducted with *Picea taeda* although *Pinus pinaster*, *Pinus radiata*, *Picea abies*, *Picea glauca* and *Chamaecyparis obtusa* have also been examined. Target gene studies began in this research area with the reporting of the involvement of 4-coumarate:coenzyme A ligase (4CL) during CW formation (Zhang and Chiang 1997). Along with up-regulation of *4CL* transcripts, the corresponding enzyme activity was also increased and its impact on lignin composition was observed. Regulators of lignin biosynthesis have been targeted as well, namely MYB factors in *P. glauca* (Bedon et al. 2007).

Meanwhile, transcriptome profiling started with the pioneering work showing *Pinus taeda* transcripts down-regulated for genes involved in lignin biosynthesis (Allona et al. 1998) and later in *C. obtusa* (Yamashita et al. 2008). At the same time, a large diversity of responses was reported for carbohydrates enzymes, i.e. xyloglucan endo-transglycosylase (XET), in CW using 1,097 ESTs in a co-expression clustering study (Allona et al. 1998). AGPs were reported as key-players through cDNAs encoding six novel so-called cell wall-associated proteins in CW formation by the same approach (Zhang et al. 2000). These results were further developed with a set of 2,400 ESTs from a cDNA microarray where 33 out of 69 transcripts were differential in CW and related to monolignols biosynthesis (Whetten et al. 2001). Following this, studies on angiosperms provided additional information by deciphering the gelatinous layer (G-layer) deposition in the so-called G-fibres during TW formation. Fasciclin-like arabinogalactan (FLA) proteins and their corresponding transcripts have been intensively studied emphasizing their involvement as a hypothetical adhesion factor facilitating cellulose deposition in the G-layer during TW formation in *P. tremula* × *tremuloides* (Andersson-Gunnerås et al. 2003, 2006), *P. tremula* × *alba* (Lafarguette et al. 2004), *E. grandis* (Qiu et al. 2008), *E. nitens* and *A. thaliana* (MacMillan et al. 2010). Along with FLA studies, transcriptional mechanisms of saccharide metabolism and deposition were also functionally dissected, even down to the cell scale using microgenomic tools (Goué et al. 2008). Major works outlined the role of sucrose synthase (SuSy) in *P. tremula* × *alba* (Déjardin et al. 2004), cellulose synthase (CesA) in *E. globulus* (Paux et al. 2005), XET and xylo-glucan endo-transglycosylase/hydrolase (XTH) in *P. tremula* × *tremuloides*, *Populus alba* and *P. tremula* (Nishikubo et al. 2007) and XET in *L. tulipifera* (Jin et al. 2011).

With the improvement of sequencing facilities and transcriptome-wide studies, development of collections of ESTs related to wood formation transcriptomics were set-up, some of them including data related to RW formation as for *P. tremula* × *tremuloides* (Sterky et al. 2004), but also including expression data in RW for several other poplar species (Sjodin et al. 2009), for *P. abies* (Koutaniemi et al. 2007), for *P. radiata* (Ramos et al. 2012) and for *P. pinaster* (Villalobos et al. 2012).

A striking point with timescale studies of RW formation is that very few reports deal with early molecular events of the process. In other words, most of the published works dealing with transcriptomics of RW formation, either gene-targeted or genome-wide, focus at developmental stages when RW is already histologically observable in the xylem. Precursor work in the field of the induction

of RW does exist but is only gene-targeted at the moment. ZFP2 transcription factor was firstly reported in *Juglans regia* (Leblanc-Fournier et al. 2008) and *P. tremula* × *alba* (Martin et al. 2009). This ZFP2 is coined "mechano-sensitive" and addresses the xylem cell response to mechanical stress at a very early stage in a timely and structured manner in the transduction pathway to TW formation in trees, along with TCH2 and TCH4 as reported in *P. tremula* × *alba* from quantitative PCR studies (Martin et al. 2010). TCH4, reported as encoding for an XET in *A. thaliana* (Xu et al. 1995), draws attention to enzymatic-oriented cellulose modifications in the cell wall. The field of early RW induction, at the cell level and before any macroscopic tissue organization can be observed in the stem, is hopefully a must in any forthcoming experiments.

4.4.2 Techniques for Proteome Measurement

Proteomics is a powerful molecular tool for describing proteomes at the organelle, cell, organ or tissue levels and for showing the modifications of protein expression in response to environmental changes (Abbasi and Komatsu 2004). Proteomics completes the large-scale analysis of the transcriptome. On many occasions, the level of mRNA is not always correlated with protein expression level. One transcript may be translated into more than one protein because of alternative splicing or alternative post-translational modifications. In addition, post-translational modifications such as phosphorylation and glycosylation may modify protein activities and subcellular localization (Yan et al. 2005).

Although attempts have been made at identifying proteins whose abundance, localization, and/or post-transcriptional modifications are altered by gravistimulation, most studies were conducted on *A. thaliana* seedlings and tended to unravel the mechanisms that control root gravitropism (for review, see Harrison et al. 2008). As for the understanding of the response of tree shoots to gravity, both gymnosperm and angiosperm species should be considered separately since the structure and properties of CW are different from those of TW. Although studies have been conducted to elucidate wood formation in trees, few of them have addressed the problem at the proteomic level, and even less focussed on RW genesis.

Among the first global attempts to unravel xylogenesis in trees, two-dimensional (2D) electrophoresis has been used to characterize xylem maritime pine proteins (Costa et al. 1999) or seasonal changes in protein expression in wood-forming tissues of poplar (Minsbrugge et al. 2000). The first description of the proteome of maritime pine wood-forming tissue (identification of 175 proteins) was provided by Gion et al. (2005). The variations in the proteome of differentiating xylem collected from the top to the bottom of adult maritime pine (*P. pinaster*) trees have provided a list of candidate genes for wood properties (Paiva et al. 2007). Using a large-scale approach, regeneration of the secondary vascular system in poplar was studied after peeling of the bark and sampling by scraping regenerating tissues (Juan et al. 2006).

A dynamic view of the changes occurring during the juvenile wood formation in the proteome of *E. grandis* has been provided using xylem tissues from 3- and 6-year-old trees (Celedon et al. 2007). More recently, a focussed analysis of plasma membrane proteomes from different tissues isolated from 3 to 4 m high poplar trees identified 108 proteins that were specifically expressed in the xylem (Nilsson et al. 2010). The authors proposed a schematic model for wood formation, integrating proteins expressed in the xylem such as cellulose-synthesizing complex, receptors, glucan sythase, AGPs, and enzymes of lignin biosynthesis. In particular the thorough investigation of cellulose synthase complexes in differentiating *Populus* xylem has been realized using complementary approaches including laser microdissection, immunolocalization along with proteomic analysis (Song et al. 2010).

With the aim of understanding TW induction or formation, proteomic analyses have been conducted on *Poplar* and *Eucalyptus*. These species are used as models in forest genetics and woody plant studies because they grow rapidly, they can be genetically transformed and the size of their genome is relatively small (5 to 6×10^8 bp) (Plomion et al. 2001). As for CW, different pine species, Sitka spruce (*Picea sitchensis*) and Japanese cypress (*C. obtusa*) have been used because of their economic and ecological interest. To investigate differentially expressed proteins in response to gravity, most studies report 2D polyacrylamide gel electrophoresis (PAGE) patterns and include identification of proteins by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), mass spectrometry (MS) or by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). One study using Multidimensional Protein Identification Technology (MudPIT) reported on the proteome of *Populus* developing xylem (Kalluri et al. 2009). The proteins were extracted from subcellular fractions of xylem stems, enzymatically digested and the resulting peptides were analysed using LC-MS/MS. However, this study was not specifically addressing RW induction/formation. In the next paragraphs, proteomic studies on TW induction/formation are first presented, followed by data on CW. Depending on the studies, the gravistimulation design, the organs and tissues used for protein extraction have been very different. In addition, studies on TW formation after bending using constraining strings are also presented. Because of the diversity of experimental designs, the synthesis of the results remains problematic.

4.4.3 Measured Changes in Proteome

Azri et al. (2009) studied young poplars (14–20 internodes) inclined at 35° from the vertical axis. Whole internodes from the basal and apical regions of vertical and gravistimulated stems were collected. The purpose of this experimental procedure was to allow analysis of the differential expression caused by gravistimulation between regions showing different motors for stem reorientation. The apical region responds to inclination by differential growth due to elongation of primary tissues

while reorientation is due to asymmetrical formation of RW at the base. After 45 min of gravistimulation, the stem showed no reorientation. After 1 week, RW was observed at the base of the stem. Differential protein expression was reported between inclined or non-inclined conditions and also between the regions of the stem. Among 300 protein spots, 40 % showed significant changes after inclination. Sixty protein spots whose staining intensity was altered by gravistimulation were identified by mass spectrometry. These 60 proteins fell into a large range of functional categories. Interestingly, the patterns of expression of these selected proteins differed strongly between the conditions tested (apical and basal regions, 45 min and 1 week of inclination). At 45 min and 1 week, respectively, three and four proteins were similarly regulated by gravistimulation between the top and the basal regions. These observations suggested that different metabolisms and signaling pathways were involved in each region of the stem following a short (45 min) or a long (1 week) exposure to gravistimulation. At 45 min, before any visible reorientation of the stem, some of the proteins regulated by gravistimulation may be involved in graviperception. At the top of the stem (where reorientation will later occur through differential elongation of primary tissues), the results suggested the implication of ROS (regulation of oxidative stress-responsive enzymes). The regulation of actin and tubulin subunits, or microtubule-binding proteins showed the importance of cell wall—plasma membrane—cytoskeleton structural continuum for graviperception. Several proteins suggested some signaling via the endomembrane system and that calcium and phosphoinositides might act as cellular messengers (calreticulin, phosphatidyl inositol transfer protein SEC14). At the base of the stem (where orientation will later occur through the formation of RW), the most noticeable enzymes that were differentially expressed by gravistimulation were involved in lignin biosynthesis (phenylcoumaran benzylic ether reductase, S-adenosylmethionine synthase). However, members of the S-adenosyl-L-methionine-synthase gene family, which serve as universal methyl-group donors, are potentially involved in lignin as well as in ethylene biosynthesis pathways.

In *Eucalyptus gunnii*, proteins were extracted from xylem tissue harvested from a crooked tree. Two-dimensional gel electrophoresis images from normal and TW were compared showing that 12 proteins out of 140 proteins analysed were differentially expressed (Plomion et al. 2003). However, none of these proteins were identified.

A different approach was carried out by Kaku et al. (2009) who focused on the proteome of the G-layer in poplar TW. Leaning stems and branches from field-grown poplars were used as sources for isolation of G-layers from TW. Among the proteins separated by 2D gel electrophoresis, 108 were identified. Most abundant were lignin synthesis-related proteins although the G layer did not contain lignin itself. Cytoskeleton proteins, methionine synthesis-related proteins and cell wall-related proteins were also identified. Lignification in TW is still a matter of debate. Andersson-Gunnerås et al. (2006) using a global analysis reported a decrease in monolignol biosynthesis in TW compared with normal wood. However, on-going lignification was observed during G layer deposition in the compound middle lamella, S1 and S2 layers in poplar TW (Yoshinaga et al. 2012). An assay based

on protein cleavage isotope dilution mass spectroscopy (PC-IDMS) has been developed for quantification of proteins regulating monolignol biosynthesis in *P. trichocarpa* (Shuford et al. 2012) and could potentially bring valuable data to decipher lignification in RW.

In conifers, CW is formed in response to gravitropic stimulus or environmental disturbances such as prevailing winds, and "pushes" the stems toward a vertical orientation. In the same way as for the TW studies, proteomic analyses of CW formation concerned either developing CW or inclined stems where no CW had been formed yet.

A comparative protein-based approach to identify proteins specifically expressed in CW was conducted with branches of Sitka spruce (*P. sitchensis*) (McDougall 2000). The developing xylem was sampled from the compression and non-compression sides of the branches. The comparison of polypeptides patterns by SDS-PAGE led to the identification of a laccase-type polyphenol oxidase that was over-expressed in compression tissues. This enzyme is thought to be involved in lignin biosynthesis.

On a larger scale, the identification of CW responsive proteins has been conducted with a 22-year-old crooked maritime pine (*P. pinaster* Ait.) (Plomion et al. 2000). Wood samples were mechanically and chemically characterized by measuring growth strains and lignin and cellulose contents, respectively. Of the 137 spots studied, 19 % were associated with growth strain effect. The results indicated the importance of ethylene in CW formation. The implication of 1-aminocyclopropane-1-carboxylate (ACC) oxidase which catalyses the final reaction of the ethylene biosynthetic pathway in CW formation has also been suggested by Yuan et al. (2010). These authors examined PtACO1 and *PtACO1*-like (encoding putative ACC oxidases) transcript levels by quantitative PCR in loblolly pine seedling stems that were bent to a 90° angle using constraining strings. They observed an increase in these transcripts levels starting at 30 min and peaking at 3 h after bending. *PtACO1*-like transcripts were higher in CW than in opposite wood (OW). Besides, Plomion et al. (2000) have found that lignin biosynthesis was also affected during CW formation and that enzymes involved in Krebs cycle, sucrose and starch metabolism were up-regulated.

In another study, sampling of compression and OW was done with 16-year-old maritime pines bent to a 15° angle by tying their trunks to neighbouring trees for 2 years (Gion et al. 2005). Other types of wood were also analysed (juvenile and mature woods, early and late woods). The clustering of 215 proteins identified over the six types of wood was presented. It appeared that 20 % of the identified proteins exhibited distinctive expression patterns between CW and OW. Profilin, actin and nucleoside diphosphate kinase, 40S ribosomal S12 proteins were under-expressed in CW.

LC-MS analysis of Golgi-enriched membrane fraction from developing *P. radiata* CW has been done following in-solution digestion with trypsin (Mast et al. 2010). CW was sampled from 6-year-old trees in late summer to maximize the identification of proteins involved in secondary cell wall formation. As expected most proteins detected were involved not only in cell wall synthesis (i.e. cellulose

synthase, laccase, phenyl alanine ammonia-lyase) but also in hormone biosynthesis and signaling (i.e. auxin-induced proteins, ACC synthase) and stress and defence response. Within this last putative functional category, numerous receptors were found (CC-NBS-LRR protein, NBS/LRR, TIR/NBS, TIR/NBS/LRR disease resistance protein).

Gravitropism is not the only process that determines stem orientation; phototropism is also an important factor that can lead to RW production. The interaction between these two processes has not been extensively studied in trees. Herrera et al. (2010) have presented a proteomic analysis of inclined pine seedlings submitted to an orthogonal light source. However, the apices were collected instead of the basal part of the stem where undergoing secondary growth takes place. Thus this study mainly identified differentially expressed proteins in the primary response to stem tilting.

Proteomic studies have been realized with different organs and tissues (stem, branches, whole internodes, xylem, G layer) from seedlings to trees aged from 2 month-old to 22 year-old. In some cases the plants were inclined or mechanically bent and the proteome was analysed after varying times depending on the study (from 45 min to days or weeks). In other cases, aged plants showing RW were used. The problem is to discriminate between overlapping events such as induction, signal transduction (first events following stimulation) in the stimulated cells which are not clearly identified yet, reorientation of cambial cell programming, and differentiation of newly formed cells in the developing xylem. In addition, perception of the gravitropic stimulus and response probably occur in different cells. The proteomic approach has been executed either on whole internodes or on xylem tissue which may not contain the perceptive cells. Among the proteins listings published, large functional categories appear such as primary metabolism, cytoskeleton organization and biogenesis, cell wall synthesis, hormone biosynthesis and signaling. However, the role of most proteins is still hypothetical. Focused studies are needed to evaluate the role of the proteins brought forward by global proteomic analyses.

4.4.4 Metabolomics and RW Formation

Metabolomics is a global approach used in biology for systematic metabolite quantification, a metabolite being any intermediate or product of the metabolism, e.g. amino acids, carbohydrates, hormones, and many more. Although several studies targeted some metabolites related to carbon and secondary metabolism that potentially play a role in RW formation (Yeh et al. 2005, 2006; Shi and Li 2012), "without *a priori*" approaches have been marginally exploited in this field. To date, only Andersson-Gunnerås et al. (2006) have published work using this technique. A combination of metabolomics and transcriptomics recently gave precious insights on the *Arabidopsis* gravitropism and phototropism interplay (Millar and Kiss 2013). Andersson-Gunnerås et al. (2006) used a similar approach

to gain access to the G-layer formation in poplar TW induced after 11 days bending. Conclusions from biochemical measurements follow predictions coherent with gene expression showing in TW: an increased activity of cellulose synthesis and pectin degradation-related genes while those involved in lignin biosynthesis are decreased. An advantage of their transcriptomic approach consists in fine identification of differentially expressed genes from multigenic families, thus refining the implication of gene candidates individually. Based on their expression and metabolic profiles, they propose extensive relational models for carbon metabolism and lignin biosynthesis in TW. Despite the importance of this work that provides a coherent framework based on quantitative and qualitative data on TW chemistry and gene expression, the earlier steps remain a matter of discovery.

4.5 Concluding Remarks

The formation of RW allows woody structures to adapt their position in response to gravitational and mechanical stimulation and/or a change in the light environment. The deciphering of the molecular mechanisms underlying this particular growth response is complex. It requires at the very least, tree models with sequenced genomes, which allow global approaches such as transcriptomics and proteomics. Functional genomics which aims to elucidate the function of proteins encoded by candidate genes is limited by a scarcity of mutants and the long generation times of forest trees. In the face of these complex challenges, Wyatt et al. (2010) presented *A. thaliana* as a model for a molecular and genetic analysis of the mechanisms of TW formation. In addition, RW formation having many external or internal causal agents (gravity, light, interactions of both stimuli, inherent patterning mechanisms), it is difficult to set up an experimental design that addresses the impact of one particular stimulus in trees. Most studies utilized inclined trees in greenhouse conditions, although both phototropic and gravitropic reactions occurred in such conditions. Signalisation pathways leading to tree stature adjustments are different whether starting from a light or a gravi-mechanical stimulus. For a short period of time, dark or isotropic light conditions could be used to gain insight into gravitropic signalisation pathway leading to RW formation. Moreover the issues around staking also need to be considered since different molecular pathways may be induced if stem deformation is allowed or not.

Hypotheses about perception of gravistimulation were previously defined through studies using *A. thaliana* mutants. However, in trees the question about the role of amyloplasts remains since starch is present in high level and everywhere in old woody structures. In trees, the tissue/cell that perceives gravistimulation is not clearly identified. As for the early events of signal transduction, one has to emphasize that very few studies were done at the very beginning of induction of RW, before any macroscopic observation of RW formation. Mechano-receptors involved in RW induction have still to be characterized. Global approaches suggest the role of calcium and ROS as second messengers and some signaling via the

endomembrane system and phosphoinositides. Although components of the signaling network have been identified through global analyses, the way they relate to one another in space and time is still unknown. Wyatt and Kiss (2013) speak about a "more or less amorphous gray cloud" when relating to the understanding of the signaling network. More precision could come from microdissection of chosen tissues or cells prior to molecular investigations. Typically, the early events of signal transduction are supposed to lead to hormonal response (i.e. synthesis, degradation, redistribution, reallocation, compartmentalization, and so on) that will finally provoke the growth and cell differentiation response. The involvement of ethylene gibberellin and auxin has been discussed but more studies are needed in order to decipher hormone signaling crosstalk in RW induction at the cell level, and also at the organ and whole plant level. For example, Azri et al. (2009) suggested that different signaling pathways occurred at the top and the base of a tilted poplar stems.

In conclusion, global approaches reveal the complexity of the RW induction both on a temporal scale and as a function of the location in the tree. Therefore, although the transcriptional network, the organization of protein synthesis and the subsequent hormonal response at the whole tree level is still unknown, the beginning of an understanding of how trees manipulate RW formation to solve their mechanical requirements is emerging.

References

Abbasi FM, Komatsu S (2004) A proteomic approach to analyze salt-responsive proteins in rice leaf sheath. Proteomics 4:2072–2081

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in plant biology, 2nd edn. Academic, San Diego

Allen GJ, Muir SR, Sanders D (1995) Release of Ca^{2+} from individual plant vacuoles by both InsP3 and cyclic ADP-ribose. Science 268:735–737

Allona I, Quinn M, Shoop E, Swope K, Saint Cyr S, Carlis J, Riedl J, Retzel E, Campbell MM, Sederoff R, Whetten RW (1998) Analysis of xylem formation in pine by cDNA sequencing. Proc Natl Acad Sci U S A 95:9693–9698

Almeras T, Fournier M (2009) Biomechanical design and long-term stability of trees: morphological and wood traits involved in the balance between weight increase and the gravitropic reaction. J Theor Biol 256:370–381

Almeras T, Costes E, Salles JC (2004) Identification of biomechanical factors involved in stem shape variability between apricot tree varieties. Ann Bot 93:455–468

Andersson-Gunneråas S, Hellgren JM, Björklund S, Regan S, Moritz T, Sundberg B (2003) Asymmetric expression of a poplar ACC oxidase controls ethylene production during gravitational induction of tension wood. Plant J 34:339–349

Andersson-Gunneråas S, Mellerowicz EJ, Love J, Segerman B, Ohmiya Y, Coutinho PM, Nilsson P, Henrissat B, Moritz T, Sundberg B (2006) Biosynthesis of cellulose-enriched tension wood in *Populus*: global analysis of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. Plant J 45:144–165

Azri W, Chambon C, Herbette S, Brunel N, Coutand C, Leplé JC, Ben Rejeb I, Ammar S, Julien JL, Roeckel-Drevet P (2009) Proteome analysis of apical and basal regions of poplar stems under gravitropic stimulation. Physiol Plant 136:193–208

Azri W, Brunel N, Franchel J, Ben Rejeb I, Jacquot JP, Julien J-L, Herbette S, Roeckel-Drevet P (2013) Putative involvement of thioredoxin *h* in early response to gravitropic stimulation of poplar stems. J Plant Physiol 170:707–711

Baba K, Adachi K, Take T, Yokoyama T, Ito T, Nakamura T (1995) Induction of tension wood in GA_3-treated branches of the weeping type of Japanese cherry, *Prunus spachiana*. Plant Cell Physiol 36:983–988

Baluška F, Volkmann D (2011) Mechanical aspects of gravity-controlled growth, development and morphogenesis. In: Wojtaszek P (ed) Mechanical integration of plant cells and plants, Signaling and communication in plants. Springer-Verlag GmbH, Heidelberg, pp 195–222

Baluška F, Šamaj J, Wojtaszek P, Volkmann D, Menzel D (2003) Cytoskeleton-plasma membrane-cell wall continuum in plants. Emerging links revisited. Plant Physiol 133:482–491

Bastien R, Bohr T, Moulia B, Douady S (2013) Unifying model of shoot gravitropism reveals proprioception as a central feature of posture control in plants. Proc Natl Acad Sci U S A 110:755–760

Bedon F, Grima-Pettenati J, Mackay J (2007) Conifer R2R3-MYB transcription factors: sequence analyses and gene expression in wood-forming tissues of white spruce (*Picea glauca*). BMC Plant Biol 7:17

Berthier S, Stokes A (2005) Phototropic response induced by wind loading in Maritime pine seedlings (*Pinus pinaster* Ait.). J Exp Bot 56:851–856

Björklund S, Antti H, Uddestrand I, Moritz T, Sundberg B (2007) Cross-talk between gibberellin and auxin in development of *Populus* wood: gibberellin stimulates polar auxin transport and has a common transcriptome with auxin. Plant J 52:499–511

Booth IR, Blount P (2012) The MscS and MscL families of mechanosensitive channels act as microbial emergency release valves. J Bacteriol 194:4802–4809

Braam J (2005) In touch: plant responses to mechanical stimuli. New Phytol 165:373–389

Celedon PAF, de Andrade A, Xavier Meireles KG, Gallo de Carvalho MC, Gomes Caldas DG, Moon DH, Tozelli Carneiro R, Franceschini LM, Oda S, Labate CA (2007) Proteomic analysis of the cambial region in juvenile *Eucalyptus grandis* at three ages. Proteomics 7:2258–2274

Collet C, Fournier M, Ningre F, Hounzandji AP, Constant T (2011) Growth and posture control strategies in *Fagus sylvatica* and *Acer pseudoplatanus* saplings in response to canopy disturbance. Ann Bot 107:1345–1353

Correll MJ, Kiss JZ (2002) Interactions between gravitropism and phototropism in plants. J Plant Growth Regul 21:89–101

Cosgrove DJ, Hedrich R (1991) Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. Planta 186:143–153

Costa P, Pionneau C, Bauw G, Dubos C, Bahrmann N, Kremer A, Frigerio J-M, Plomion C (1999) Separation and characterization of needle and xylem maritime pine proteins. Electrophoresis 20:1098–1108

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A (2012) Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483:176–181

Coutand C, Fournier M, Moulia B (2007) The gravitropic response of poplar trunks: key roles of prestressed wood regulation and the relative kinetics of cambial growth versus wood maturation. Plant Physiol 144:1166–1180

Decreux A, Messiaen J (2005) Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. Plant Cell Physiol 46:268–278

Déjardin A, Leplé J-C, Lesage-Descauses M-C, Costa G, Pilate G (2004) Expressed sequence tags from poplar wood tissues – a comparative analysis from multiple libraries. Plant Biol 6:55–64

Ding JP, Pickard BG (1993) Modulation of mechanosensitive calcium-selective cation channels by temperature. Plant J 3:713–720

Du S, Yamamoto F (2003) A study on the role of calcium in xylem development and compression wood formation in *Taxodium distichum* seedlings. IAWA J 24:75–85
Du S, Yamamoto F (2007) An overview of the biology of reaction wood formation. J Integr Plant Biol 49:131–143
Elo A, Immanen J, Nieminen K, Helariutta Y (2009) Stem cell function during plant vascular development. Semin Cell Dev Biol 20:1097–1106
Eriksson ME, Israelsson M, Olsson O, Moritz T (2000) Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. Nat Biotechnol 18:784–788
Felten J, Sundberg B (2013) Biology, chemistry and structure of tension wood. In: Fromm J (ed) Cellular aspects of wood formation, plant cell monographs 20. Springer, Heidelberg, pp 203–224
Fournier M, Chanson B, Thibaut B, Guitard D (1994) Measurements of residual growth strains at the stem surface observations on different species. Ann Sci For 51:249–266
Funada R, Kubo T, Fushitani M (1990) Earlywood and latewood formation in *Pinus densiflora* trees with different amounts of crown. IAWA Bull 11:281–288
Funada R, Miura T, Shimizu Y, Kinase T, Nakaba S, Kubo T, Sano Y (2008) Gibberellin-induced formation of tension wood in angiosperm trees. Planta 227:1409–1414
Gion J-M, Lalanne C, Le Provost G, Ferry-Dumazet H, Paiva J, Chaumeil P, Frigerio J-M, Brach J, Barré A, de Daruvar A, Claverol S, Bonneu M, Sommerer N, Negroni L, Plomion C (2005) The proteome of maritime pine wood forming tissue. Proteomics 5:3731–3751
Gou J, Ma C, Kadmiel M, Gai Y, Strauss S, Jiang X, Busov V (2011) Tissue-specific expression of *Populus* C_{19} GA 2-oxidases differentially regulate above- and below-ground biomass growth through control of bioactive GA concentrations. New Phytol 192:626–639
Goué N, Lesage-Descauses M-C, Mellerowicz EJ, Magel E, Label P, Sundberg B (2008) Microgenomic analysis reveals cell type-specific gene expression patterns between ray and fusiform initials within the cambial meristem of *Populus*. New Phytol 180:45–56
Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. Nucleic Acids Res 36:D154–D158
Haley ANN, Russellt AJ, Wood N, Allan AC, Knight M, Campbell AK (1995) Effects of mechanical signaling on plant cell cytosolic calcium. Proc Natl Acad Sci U S A 92:4124–4128
Harrison BR, Morita MT, Masson PH, Tasaka M (2008) Signal transduction in gravitropism. In: Gilroy S, Masson PH (eds) Plant tropisms. Blackwell Publishing, Oxford, pp 21–46
Hashiguchi Y, Tasaka M, Morita MT (2013) Mechanism of higher plant gravity sensing. Am J Bot 100:91–100
Haswell ES, Peyronnet R, Barbier-Brygoo H, Meyerowitz EM, Frachisse JM (2008) Two MscS homologs provide mechanosensitive channel activities in the *Arabidopsis* root. Curr Biol 18:730–734
Haswell ES, Phillips R, Rees DC (2011) Mechanosensitive channels: what can they do and how do they do it? Structure 19:1356–1369
He ZH, Fujiki M, Kohorn BD (1996) A cell wall-associated, receptor-like protein kinase. J Biol Chem 271:19789–19793
He ZH, Cheeseman I, He D, Kohorn BD (1999) A cluster of five cell wall-associated receptor kinase genes, *Wak1-5*, are expressed in specific organs of *Arabidopsis*. Plant Mol Biol 39:1189–1196
Hellgren JM, Olofsson K, Sundberg B (2004) Patterns of auxin distribution during gravitational induction of reaction wood in poplar and pine. Plant Physiol 135:212–220
Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Höfte H (2007) A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. Curr Biol 17:922–931
Herrera R, Krier C, Lalanne C, Ba EHM, Stokes A, Salin F, Fourcaud T, Claverol S, Plomion C (2010) (Not) keeping the stem straight: a proteomic analysis of maritime pine seedlings undergoing phototropism and gravitropism. BMC Plant Biol 10:217–229

Hohm T, Preuten T, Fankhauser C (2013) Phototropism: translating light into directional growth. Am J Bot 100:47–59
Hrabak EM, Chan CWM, Gribskov M, Harper JF, Choi JH, Halford N, Luan S, Nimmo HG, Sussman MR, Thomas M, Walker-Simmons K, Zhu JK, Harmon AC (2003) The *Arabidopsis* CDPK-SnRK superfamily of protein kinases. Plant Physiol 132:666–680
Humphrey TV, Bonetta DT, Goring DR (2007) Sentinels at the wall: cell wall receptors and sensors. New Phytol 176:7–21
Jin H, Do J, Moon D, Noh EW, Kim W, Kwon M (2011) EST analysis of functional genes associated with cell wall biosynthesis and modification in the secondary xylem of the yellow poplar (*Liriodendron tulipifera*) stem during early stage of tension wood formation. Planta 234:959–977
Jones-Rhodes MW, Bartel DP, Barterl B (2006) MicroRNAs and their regulatory roles in plants. Annu Rev Plant Biol 57:19–53
Jourez B, Avella-Shaw T (2003) Effet de la durée d'application d'un stimulus gravitationnel sur la formation de bois de tension et de bois opposé dans de jeunes pousses de peuplier (*Populus euramericana* cv 'Ghoy'). Ann For Sci 60:31–41
Juan D, Hong-Li X, De-Qiang Z, Xin-Qinag H, Min-Jie W, Ying-Zhang L, Ke-Ming C, Meng-Zhu L (2006) Regeneration of the secondary vascular system in poplar as a novel system to investigate gene expression by a proteomic approach. Proteomics 6:881–895
Kaku T, Serada S, Baba K, Tanaka F, Hayashi T (2009) Proteomic analysis of the G-layer in poplar tension wood. J Wood Sci 55:250–257
Kalluri UC, Hurst GB, Lankford PK, Ranjan P, Pelletier DA (2009) Shotgun proteome profile of *Populus* developing xylem. Proteomics 9:4871–4880
Kern VD, Sack FD (1999) Irradiance-dependent regulation of gravitropism by red light in protonemata of the moss *Ceratodon purpureus*. Planta 209:299–307
Kim SE, Coste B, Chadha A, Cook B, Patapoutian A (2012) The role of Drosophila Piezo in mechanical nociception. Nature 483:209–212
Knight MR, Campbell AK, Smith SM, Trewavas AJ (1991) Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature 352:524–526
Knight MR, Smith SM, Trewavas AJ (1992) Wind-induced plant motion immediately increases cytosolic calcium. Proc Natl Acad Sci U S A 89:4967–4971
Kojima M, Becker VK, Altaner CM (2012) An unusual form of reaction wood in Koromiko [*Hebe salicifolia* G.Forst. (Pennell)], a southern hemisphere angiosperm. Planta 235:1315–1330
Koutaniemi S, Warinowski T, Karkonen A, Alatalo E, Fossdal CG, Saranpaa P, Laakso T, Fagerstedt KV, Simola LK, Paulin L, Rudd S, Teeri TH (2007) Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. Plant Mol Biol 65:311–328
Kurusu T, Kuchitsu K, Nakano M, Nakayama Y, Iida H (2013) Plant mechanosensing and Ca^{2+} transport. Trends Plant Sci 18:227–233
Lafarguette F, Leplé J-C, Déjardin A, Laurans F, Costa G, Lesage-Descauses M-C, Pilate G (2004) Poplar genes encoding fasciclin-like arabinogalactan proteins are highly expressed in tension wood. New Phytol 164:107–121
Lally D, Ingmire P, Tong HY, He ZH (2001) Antisense expression of a cell wall-associated protein kinase, WAK4, inhibits cell elongation and alters morphology. Plant Cell 13:1317–1331
Leblanc-Fournier N, Coutand C, Crouzet J, Brunel N, Lenne C, Moulia B, Julien J-L (2008) *Jr-ZFP2*, encoding a Cys2/His2-type transcription factor, is involved in the early stages of the mechano-perception pathway and specifically expressed in mechanically stimulated tissues in woody plants. Plant Cell Environ 31:715–726
Lindner H, Müller LM, Boisson-Dernier A, Grossniklaus U (2012) CrRLK1L receptor-like kinases: not just another brick in the wall. Curr Opin Plant Biol 15:659–669
Little CHA, Savidge RA (1987) The role of plant-growth regulators in forest tree cambial growth. Plant Growth Regul 6:137–169

Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B (2009) Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. Proc Natl Acad Sci U S A 106:5984–5986

Lu S, Sun Y-H, Shi R, Clark C, Li L, Chiang VL (2005) Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*. Plant Cell 17:2186–2205

Lu S, Li L, Yi X, Joshi CP, Chiang VL (2008) Differential expression of three *Eucalyptus* secondary cell wall-related cellulose synthase genes in response to tension stress. J Exp Bot 59:681–695

MacMillan CP, Mansfield SD, Stachurski ZH, Evans R, Southerton SG (2010) Fasciclin-like arabinogalactan proteins: specialization for stem biomechanics and cell wall architecture in *Arabidopsis* and *Eucalyptus*. Plant J 62:689–703

Marín-González E, Suárez-López P (2012) "And yet it moves": cell-to-cell and long-distance signaling by plant microRNAs. Plant Sci 196:18–30

Martin L, Leblanc-Fournier N, Azri W, Lenne C, Henry C, Coutand C, Julien J-L (2009) Characterization and expression analysis under bending and other abiotic factors of *PtaZFP2*, a poplar gene encoding a Cys2/His2 zinc finger protein. Tree Physiol 29:125–136

Martin L, Leblanc-Fournier N, Julien J-L, Moulia B, Coutand C (2010) Acclimation kinetics of physiological and molecular responses of plants to multiple mechanical loadings. J Exp Bot 61:2403–2412

Mast S, Peng L, Jordan W, Flint H, Phillips L, Donaldson L, Strabala TJ, Wagner A (2010) Proteomic analysis of membrane preparations from developing *Pinus radiata* compression wood. Tree Physiol 30:1456–1468

Matsuzaki J, Masumori M, Tange T (2006) Stem phototropism of trees: a possible significant factor in determining stem inclination on forest slopes. Ann Bot 98:573–581

Matsuzaki J, Masumori M, Tange T (2007) Phototropic bending of non-elongating and radially growing woody stems results from asymmetrical xylem formation. Plant Cell Environ 30:646–653

Mauriat M, Moritz T (2009) Analyses of *GA20ox*- and *GID1*-over-expressing aspen suggest that gibberellins play two distinct roles in wood formation. Plant J 58:989–1003

McDougall GJ (2000) A comparison of proteins from the developing xylem of compression and non-compression wood of branches of Sitka spruce (*Picea sitchensis*) reveals a differentially expressed laccase. J Exp Bot 51:1395–1401

Mellerowicz EJ, Immerzeel P, Hayashi T (2008) Xyloglucan: the molecular muscle of trees. Ann Bot 105:659–665

Millar KD, Kiss JZ (2013) Analyses of tropistic responses using metabolomics. Am J Bot 100:79–90

Minsbrugge KV, Meyermans H, Van Montagu M, Bauw G, Boerjan W (2000) Wood formation in poplar: identification, characterization, and seasonal variation of xylem proteins. Planta 210:589–598

Monshausen GB, Haswell ES (2013) A force of nature: molecular mechanisms of mechanoperception in plants. J Exp Bot. doi:10.1093/jxb/ert204 (Epub ahead of print)

Mori IC, Schroeder JI (2004) Reactive oxygen species activation of plant Ca^{2+} channels. Signaling mechanisms in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction. Plant Physiol 135:702–708

Morita MT (2010) Directional gravity sensing in gravitropism. Annu Rev Plant Biol 61:705–720

Morita MT, Kato T, Nagafusa K, Saito C, Ueda T, Nakano A, Tasaka M (2002) Involvement of the vacuoles of the endodermis in the early process of shoot gravitropism in *Arabidopsis*. Plant Cell 14:47–56

Moulia B, Fournier M (2009) The power and control of gravitropic movements in plants: a biomechanical and systems biology view. J Exp Bot 60:461–486

Moulia B, Coutand C, Lenne C (2006) Posture control and skeletal mechanical acclimation in terrestrial plants: implications for mechanical modeling of plant architecture. Am J Bot 93:1477–1489

Moulia B, Der Loughian C, Bastien R, Martin L, Rodríguez M, Gourcilleau D, Barbacci A, Badel E, Franchel J, Lenne C, Roeckel-Drevet P, Allain JM, Frachisse JM, de Langre E, Coutand C, Leblanc-Fournier N, Julien JL (2011) Integrative mechanobiology of growth and architectural development in changing mechanical environments. In: Wojtaszek P (ed) Mechanical integration of plant cells and plants, Signaling and communication in plants. Springer-Verlag GmbH, Heidelberg, pp 269–302

Moyle R, Schrader J, Stenberg A, Olsson O, Saxena S, Sandberg G, Bhalerao RP (2002) Environmental and auxin regulation of wood formation involves members of the *Aux/IAA* gene family in hybrid aspen. Plant J 31:675–685

Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T (2007) *Arabidopsis* plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. Proc Natl Acad Sci U S A 104:3639–3644

Nakamura T (2003) Control of morphogenesis of woody plant by gravity on earth. Biol Sci Space 17:144–148

Nakamura T, Saotome M, Ishiguro Y, Itoh R, Higurashi S, Hosono M, Ishii Y (1994) The effects of GA_3 on weeping of growing shoots of the Japanese cherry, *Prunus spachiana*. Plant Cell Physiol 35:523–527

Nieminen K, Robischon M, Immanen J, Helariutta Y (2012) Towards optimizing wood development in bioenergy trees. New Phytol 194:46–53

Nilsson R, Bernfur K, Gustavsson N, Bygdell J, Wingsle G, Larsson C (2010) Proteomics of plasma membranes from poplar trees reveals tissue distribution of transporters, receptors, and proteins in cell wall formation. Mol Cell Proteomics 9:368–387

Nishikubo N, Awano T, Banasiak A, Bourquin V, Ibatullin F, Funada R, Brumer H, Teeri TT, Hayashi T, Sundberg B, Mellerowicz EJ (2007) Xyloglucan endo-transglycosylase (XET) functions in gelatinous layers of tension wood fibers in poplar—a glimpse into the mechanism of the balancing act of trees. Plant Cell Physiol 48:843–855

Nugroho WD, Yamagishi Y, Nakaba S, Fukuhara S, Begum S, Marsoem SN, Ko JH, Jin HO, Funada R (2012) Gibberellin is required for the formation of tension wood and stem gravitropism in *Acacia mangium* seedlings. Ann Bot 110:887–895

Nugroho WD, Nakaba S, Yamagishi Y, Begum S, Marsoem SN, Ko JH, Jin HO, Funada R (2013) Gibberellin mediates the development of gelatinous fibres in the tension wood of inclined *Acacia mangium* seedlings. Ann Bot 112:1321–1329. doi:10.1093/aob/mct198 (Epub ahead of print)

Osakabe Y, Kawaoka A, Nishikubo N, Osakabe K (2012) Responses to environmental stresses in woody plants: key to survive and longevity. J Plant Res 125:1–10

Paiva JAP, Gracès M, Alves A, Garnier-Géré P, Rodrigues JC, Lalanne C, Porcon S, Le Provost G, da Silva Perez D, Brach J, Frigerio J-M, Claverol S, Barré A, Fevereiro P, Plomion C (2007) Molecular and phenotypic profiling from the base to the crown in maritime pine wood-forming tissue. New Phytol 178:283–301

Paux E, Carocha V, Marques C, Mendes de Sousa A, Borralho N, Sivadon P, Grima-Pettenati J (2005) Transcript profiling of *Eucalyptus* xylem genes during tension wood formation. New Phytol 167:89–100

Perera IY, Heilmann I, Chang SC, Boss WF, Kaufman PB (2001) A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. Plant Physiol 125:1499–1507

Pilate G, Chabbert B, Cathala B, Yoshinaga A, Leple' J-C, Laurans F, Lapierre C, Ruel K (2004) Lignification and tension wood. C R Biol 327:889–901

Pivetti CD, Yen MR, Miller S, Busch W, Tseng YH, Booth IR, Saier MH (2003) Two families of mechanosensitive channel proteins. Microbiol Mol Biol Rev 67:66–85

Plieth C, Trewavas AJ (2002) Reorientation of seedlings in the earth's gravitational field induces cytosolic calcium transients. Plant Physiol 129:786–796

Plomion C, Pionneau C, Brach J, Costa P, Baillères H (2000) Compression wood-responsive proteins in developing xylem of maritime pine (*Pinus pinaster* Ait.). Plant Physiol 123:959–969
Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. Plant Physiol 127:1513–1523
Plomion C, Pionneau C, Baillères H (2003) Analysis of protein expression along the normal to tension wood gradient in *Eucalyptus gunnii*. Holzforschung 57:353–358
Pruyn ML (1997) Thigmomorphogenesis: responses of two *Populus* hybrids to mechanical stress. MSc. Thesis, Michigan State University, East Lansing, MI, 90 p
Pruyn ML, Ewers BJ, Telewski FW (2000) Thigmomorphogenesis: changes in the morphology and mechanical properties of two *Populus* hybrids in response to mechanical perturbation. Tree Physiol 20:535–540
Qin SY, Hu D, Matsumoto K, Takeda K, Matsumoto N, Yamaguchi Y, Yamamoto K (2012) Malectin forms a complex with ribophorin I for enhanced association with misfolded glycoproteins. J Biol Chem 287:38080–38089
Qiu D, Wilson IW, Gan S, Washusen R, Moran GF, Southerton SG (2008) Gene expression in *Eucalyptus* branch wood with marked variation in cellulose microfibril orientation and lacking G-layers. New Phytol 179:94–103
Ramos P, Le Provost G, Gantz C, Plomion C, Herrera R (2012) Transcriptional analysis of differentially expressed genes in response to stem inclination in young seedlings of pine. Plant Biol 14:923–933
Savidge RA, Mutumba GM, Heald JK, Wareing PF (1983) Gas chromatography-mass spectroscopy identification of 1-aminocyclopropane-1-carboxylic acid in compression wood vascular cambium of *Pinus contorta* Dougl. Plant Physiol 71:434–436
Serpe MD, Nothnagel EA (1995) Fractionation and structural characterization of arabinogalactan-proteins from the cell wall of rose cells. Plant Physiol 109:1007–1016
Shi J, Li J (2012) Metabolites changes in inclined stem. BioResources 7:3463–3475
Shiu SH, Bleecker AB (2001) Plant receptor-like kinase gene family: diversity, function, and signaling. Sci STKE 2001:re22
Shuford CM, Li Q, Sun Y-H, Chen H-C, Wang J, Shi R, Sederoff RR, Chaing VL, Muddiman DC (2012) Comprehensive quantification of monolignol-pathway enzymes in *Populus trichocarpa* by protein cleavage isotope dilution mass spectrometry. J Proteome Res 11:3390–3404
Sierra de Grado R, Pando V, Martinez-Zurimendi P, Penalvo A, Bascones E, Moulia B (2008) Biomechanical differences in the stem straightening process among *Pinus pinaster* provenances. A new approach for early selection of stem straightness. Tree Physiol 28:835–846
Sjodin A, Street NR, Sandberg G, Gustafsson P, Jansson S (2009) The *Populus* genome integrative explorer (PopGenIE): a new resource for exploring the *Populus* genome. New Phytol 182:1013–1025
Song D, Shen J, Li L (2010) Characterization of cellulose synthase complexes in *Populus* xylem differentiation. New Phytol 187:777–790
Spurr S, Hyvärinen M (1954) Compression wood in conifers as a morphogenetic phenomenon. Bot Rev 20:551–560
Sterky F, Bhalerao RR, Unneberg P, Segerman B, Nilsson P, Brunner AM, Charbonnel-Campaa L, Lindvall JJ, Tandre K, Strauss SH, Sundberg B, Gustafsson P, Uhlen M, Bhalerao RP, Nilsson O, Sandberg G, Karlsson J, Lundeberg J, Jansson S (2004) A *Populus* EST resource for plant functional genomics. Proc Natl Acad Sci U S A 101:13951–13956
Strohm AK, Baldwin KL, Masson PH (2012) Multiple roles for membrane-associated protein trafficking and signaling in gravitropism. Front Plant Sci 3:274
Telewski FW (1989) Structure and function of flexure wood in *Abies fraseri*. Tree Physiol 5:113–122
Telewski FW (2006) A unified hypothesis of mechanoperception in plants. Am J Bot 93:1466–1476

Thibaut B, Gril J, Fournier M (2001) Mechanics of wood and trees: some new highlights for an old story. Comptes Rendus de l'Académie des Sciences Serie II Fascicule B-Mécanique 329:701–716
Timell TE (1986) Compression wood in gymnosperms. Springer, Berlin, 2150 p
Toyota M, Gilroy S (2013) Gravitropism and mechanical signaling in plants. Am J Bot 100:111–125
Toyota M, Furuichi T, Tatsumi H, Sokabe M (2008) Cytoplasmic calcium increases in response to changes in the gravity vector in hypocotyls and petioles of *Arabidopsis* seedlings. Plant Physiol 146:505–514
Toyota M, Ikeda N, Sawai-Toyota S, Kato T, Gilroy S (2013) Amyloplast displacement is necessary for gravisensing in *Arabidopsis* shoots as revealed by a centrifuge microscope. Plant J. doi:10.1111/tpj.12324
Ursache R, Nieminen K, Helariutta Y (2013) Genetic and hormonal regulation of cambial development. Physiol Plant 147:36–45
Vahala J, Felten J, Love J, Gorzsás A, Gerber L, Lamminmäki A, Kangasjärvi J, Sundberg B (2013) A genome-wide screen for ethylene-induced ethylene response factors (ERFs) in hybrid aspen stem identifies ERF genes that modify stem growth and wood properties. New Phytol. doi:10.1111/nph.12386
Verica JA, He ZH (2002) The cell wall-associated kinase (*WAK*) and *WAK-like* kinase gene family. Plant Physiol 129:455–459
Villalobos D, Diaz-Moreno S, Said E-S, Canas R, Osuna D, Van Kerckhoven SH, Bautista R, Claros M, Canovas F, Canton F (2012) Reprogramming of gene expression during compression wood formation in pine: coordinated modulation of S-adenosylmethionine, lignin and lignan related genes. BMC Plant Biol 12:100
Vinterhalter D, Vinterhalter B, Orbovic V (2012) Photo- and gravitropic bending of potato plantlets obtained in vitro from single-node explants. J Plant Growth Regul 31:560–569
Wagner TA, Kohorn BD (2001) Wall-associated kinases are expressed throughout plant development and are required for cell expansion. Plant Cell 13:303–318
Whetten R, Ying-Hsuan S, Zhang Y, Sederoff R (2001) Functional genomics and cell wall biosynthesis in loblolly pine. Plant Mol Biol 47:275–291
Wilson BF, Archer RR (1977) Reaction wood: induction and mechanical action. Annu Rev Plant Physiol 28:23–43
Wilson BF, Chien CT, Zaerr JB (1989) Distribution of endogenous indole-3-acetic acid and compression wood formation in reoriented branches of Douglas-fir. Plant Physiol 91:338–344
Wyatt SE, Kiss JZ (2013) Plant tropisms: from Darwin to the international space station. Am J Bot 100:1–3
Wyatt SE, Sederoff R, Flaishman MA, Lev-Yadun S (2010) *Arabidopsis thaliana* as a model for gelatinous fiber formation. Russ J Plant Physiol 57:363–367
Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J (1995) *Arabidopsis* TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell 7:1555–1567
Yamashita S, Yoshida M, Yamamoto H, Okuyama T (2008) Screening genes that change expression during compression wood formation in *Chamaecyparis obtusa*. Tree Physiol 28:1331–1340
Yan S, Tang Z, Su W, Sun W (2005) Proteomic analysis of salt-responsive proteins in rice root. Proteomics 5:235–244
Yeh TF, Goldfarb B, Chang HM, Peszlen I, Braun JL, Kadla JF (2005) Comparison of morphological and chemical properties between juvenile wood and compression wood of loblolly pine. Holzforschung 59:669–674
Yeh TF, Morris CR, Goldfarb B, Chang HM, Kadla JF (2006) Utilization of polar metabolite profiling in the comparison of juvenile wood and compression wood in loblolly pine (*Pinus taeda*). Tree Physiol 26:1497–1503

Yoshida M, Nakamura T, Yamamoto H, Okuyama T (1999) Negative gravitropism and growth stress in GA_3-treated branches of *Prunus spachiana* Kitamura f. *Spachiana* cv. *Plenarosea*. J Wood Sci 45:368–372

Yoshinaga A, Kusumoto H, Laurans F, Pilate G, Takabe K (2012) Lignifications in poplar tension wood lignified cell wall layers. Tree Physiol 32:1129–1136

Yuan S, Wang Y, Dean JFD (2010) ACC oxidase genes expressed in the wood-forming tissues of loblolly pine (*Pinus taeda* L.) include a pair of nearly identical paralogs (NIPs). Gene 453:24–36

Zhang XH, Chiang VL (1997) Molecular cloning of 4-coumarate:coenzyme a ligase in loblolly pine and the roles of this enzyme in the biosynthesis of lignin in compression wood. Plant Physiol 113:65–74

Zhang Y, Sederoff R, Allona I (2000) Differential expression of genes encoding cell wall proteins in vascular tissues from vertical and bent loblolly pine trees. Tree Physiol 20:457–466

Zhang Z, Yu J, Li D, Zhang Z, Liu F, Zhou X, Wang T, Ling Y, Su Z (2010) PMRD: plant microRNA database. Nucleic Acids Res 38:D806–D813

Zhong R, Ye Z-H (2013) Transcriptional regulation of wood formation in tree species. In: Fromm J (ed) Cellular aspects of wood formation. Springer, Heidelberg, pp 141–158